markers predicting the chondrogenic differentiation potential of culture expanded MSCs derived from multiple donors are lacking. The objective of this study is to identify such prognostic markers.
Methods: In this study 20 human MSC donors were tested for their ability to produce cartilage in a standard chondrogenic differentiation assay consisting of pellet culture in the presence of serum free medium and TGFβ. Cartilage formation was scored on the basis of histological matrix formation, mRNA expression levels of chondrogenic marker genes and quantification of glycosaminoglycan deposition. Of each of these donors genome wide mRNA expression profiles were obtained using an affymetrix microarray platform before the onset of differentiation. In addition, small nucleotide RNAs were isolated for miRNA profiling using a panel of miRNAs previously implemented in chondrogenesis.
Results: Only 3 donors out of 20 were identified as donors with high chondrogenic potential, whereas 9 showed moderate and 8 low chondrogenic potential. Despite these huge differences in chondrogenic potential, genome wide mRNA profiling at the onset of differentiation showed only marginal differences between the 3 groups. In contrast, profiling of microRNAs (miRNAs) previously implemented in chondrogenesis and cartilage homeostasis showed a very distinctive pattern between good and bad performing donors. We also studied the changes in miRNA expression during a 7 day differentiation period of MSCs in pellet culture and identified *miR-210* and *miR-630* as positive regulators of chondrogenesis with *miR-630* as a potential marker for high performing donors. In contrast *miR-181* and *miR-34a*, both of which are negative regulators of chondrogenesis, were up-regulated during differentiation in bad performing donors.
Conclusions: In contrast to the marginal differences at the global mRNA level between good and bad MSC donors with respect of chondrogenic differentiation potential, screening of a panel of miRNAs previously implemented in cartilage formation showed more clear segregation between good and bad performing donors. MiRNA profiling of MSC donors may, therefore, have prognostic value to select MSC donors with respect of their chondrogenic differentiation potential and their capacity to restore cartilage homeostasis after intra-articular injection in the osteoarthritic joint.

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THE REPAIR OF FOCAL CARTILAGE DEFECTS USING HUMAN EMBRYONIC STEM CELLS

A. Cheng, T. Hardingham, S. Kimber. *Univ. of Manchester, Manchester, United Kingdom*

Purpose: Damage to articular cartilage occurs frequently as a result of joint trauma and disease. Cell based treatments to repair defects have been developed using autologous chondrocytes and bone marrow stem cells and have shown some success. We have investigated the potential of human embryonic stem cells (hESC) as a source of chondrocytes, as they have capacity for unlimited self-renewal and could provide a ready supply of donor cells. In initial work we developed a 14 day culture protocol using serum free, chemically defined medium and generated chondroprogenitors from hESC, which were up to 97% SOX9 positive, expressing COL2A1 and ACAN genes. This system is chemically defined and scalable and with potential to provide cells for clinical grade use. In this study we developed the protocol further and tested hESC derived chondroprogenitors in vivo in focal defects in immunocompromised nude rats.
Methods: Human embryonic stem cells (hESC) expanded on feeder culture were transferred to feeder free/ serum free culture on fibronectin coated plates. After two passages a 14 day chondrogenesis protocol was initiated with a sequential series of growth factors, which drive the hESCs through mesendoderm/mesoderm to chondroprogenitors. These cells were characterised by qRT-PCR using a range of chondrocyte specific marker genes, negative controls and by immunofluorescence for SOX9, a chondrocyte transcription factor. Some chondro-progenitors were also derived from hESC transduced with GFP using lentiviral vectors. To test their capacity for cartilage formation, chondro-progenitors in fibrin gel (3X106 cells/ml) were implanted into osteochondral defects (2mm diameter, 2mm deep, 14 defects in 8 animals) in the patella groove of nude rats. Joint tissue was removed, decalcified, fixed and sectioned for histological and immunochemical analysis after 4 weeks or 12 weeks.
Results: The hESC derived chondro-progenitors at the end of the protocol formed cell clusters and showed chondrocyte properties, including high expression of SOX9. They strongly immunostained for SOX9 protein and for collagen II and for aggrecan and expressed collagen II and XI genes, but not collagen I and negligible collagen X, which is a marker for hypertrophic chondrocytes. The expression of matrilin 3 increased and was more than 50 time higher than matrilin 1 at the end of the protocol, again suggesting an articular rather than an epiphyseal phenotype. Also, we found the expression of core band factor beta (CBF-beta) was elevated and ZNF145, ZNF219, p300 and SirT1 were also increased. Defects in nude rat joints were seeded with chondro-progenitors (approx 2 x105cells per defect) in fibrin gel and in a contralateral control, fibrin gel alone. Joint tissue was isolated at 4 weeks and 12 weeks and in these preliminary experiments histology showed evidence of repair cartilage filling in the defect areas in the cell seeded joints (cartilage in 2 from 3 animals at 4 weeks and in 2 from 4 animals at 12 weeks). When GFP cells were implanted they were detected by fluorescence within areas of neo-cartilage formation and immunohistology using anti human vimentin antibody confirmed human cells in the repair tissue, which stained with safranin O for proteoglycan and immunostained for collagen II. Only fibrous tissue was found in the defect areas of joints implanted with fibrin gel only.
Conclusions: Human embryonic stem cells in feeder free, serum free, chemically defined medium were differentiated into chondrogenic cells, which when implanted in focal defects in nude rats participated in the formation of cartilage repair tissue assessed up to 12 weeks. The study demonstrates that human embryonic stem cells can be efficiently differentiated to produce chondro-progenitors with a protocol that is suitable for future clinical applications.

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DISEASE MODIFYING, RECEPTOR SELECTIVE ANALOGS OF FGF18 FOR GLOBAL, ARTICULAR CARTILAGE PRESERVATION AND REPAIR IN EXPERIMENTAL OSTEOARTHRITIS

A. Yayon [†], D. Strauss-Ayali [†], E. Rom [†], K. Mevorat Kaplan [†], L. Niv [†], A. Blom [‡], W. van den Berg [‡]. [†] *Procore, Nes Ziona, Israel;* [‡] *Radboud Univ., Nijmegen, Netherlands*

Purpose: We aimed to study the benefits and potential adverse effects of FGF18 and FGF18 analogs on joint articular cartilage preservation in an experimental model for Osteoarthritis (OA).
Methods: A series of genetically engineered isoforms of FGF18, a major cartilage growth and differentiation factor, were tested in a murine model for surgically induced Osteoarthritis. OA was induced by transection of the anterior medial meniscotibial ligament in the knee joint, which causes destabilization. Ligands were selected for their FGF receptor specificity, discriminating FGFR2 from FGFR3 signaling in bone and cartilage. Stabilization and controlled release of the growth factors were achieved through chemical conjugation to Hyaluronic acid (HA) which were further enhanced using a novel Fibrin-HA based hydrogel. Ligands were administered by repeated intra-articular injections into the murine knee joints according to various regimens.
Results: The capacity of these factors in the preservation and tissue repair of the various articular cartilage compartments was evident by histology and gross morphology. HA link enhanced the potency of FGF18. However, wild type FGF18, particularly when administered as soluble, free ligand, significantly enhanced osteophyte formation and size at all doses tested. The soluble FGFR3 selective ligand, on the other hand, did not induce osteophytes formation. Unexpectedly, the Fibrin-HA hydrogel by itself conferred a protective effect against damage induced OA.
Conclusions: Our results demonstrate a differential effect of FGF18 analogs different in their FGF receptor activation on cartilage preservation and remodeling in a murine model of damage induced OA. More specifically we suggest that FGF18 signaling via FGFR2 may be the primary trigger for enhanced local osteogenic effect and osteophyte formation.

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LOCATION-INDEPENDANT ANALYSIS OF INTRAARTICULAR SPRIFERMIN EFFECTS ON CARTILAGE STRUCTURE USING ORDERED VALUES

S. Hellot [†], W. Wirth [‡,§], A. Guermazi [¶,‖], C. Pena Rossi [†], F. Eckstein [‡,§]. [†] *Merck Serono S.A. – Geneva (an affiliate of Merck KGaA, Darmstadt, Germany), Geneva, Switzerland;* [‡] *Paracelsus Med. Univ., Salzburg, Austria;* [§] *Chondrometrics GmbH, Ainring, Germany;* [‖] *Boston Univ., Boston, MA, USA;* [¶] *Boston Imaging Core Lab (BICL) LLC, Boston, MA, USA*

Purpose: There is a high unmet medical need for disease modifying drugs in OA that have an effect on joint structure. Fibroblast growth factor 18 (FGF18) promotes chondrocyte proliferation and stabilizes an anabolic chondrocyte phenotype. Data from a proof-of-concept trial in knee OA demonstrated that sprifermin (rhFGF18) increased total cartilage volume and thickness. This effect seemed to be more pronounced in the lateral compartment, raising the question of the distribution of the effect on cartilage change on different knee subregions. Here we use a location-independent analysis method, to identify whether sprifermin modifies the magnitude of (subregional) cartilage loss compared with placebo, independent of the location where it occurs in individual joints.
Methods: Study participants (n=168; ≥40-year-old; 69% female) had symptomatic and radiographic femorotibial OA (KLG2 or 3), and were not confined to medial disease. Sprifermin (10, 30, or 100μg) or placebo were injected once weekly for 3 weeks after randomization, and at 13 weeks. 1.5mm coronal SPGR MRIs were acquired at baseline and 13, 26 and 52 weeks. Medial (MFTC) and lateral femorotibial cartilage (LFTC) was segmented by 7 readers, with blinding to acquisition order and treatment (Chondrometrics GmbH). Cartilage thickness (changes) were computed in each of 16 femorotibial subregions (5 medial and 5 lateral tibial, 3 medial and 3 lateral femoral).The location-independent magnitude of subregional thickness changes (in mm) was calculated using ordered values (OV), individually assigning the magnitude of cartilage loss in the subregion with the greatest loss to OV1, the one with the second greatest loss to OV2, and so forth, and the one with the smallest loss/greatest increase to OV16). In the current analysis, results of the 100μg cohort (n=63) vs. matching placebo (n=21) were compared using t-tests.
Results: Total femorotibial cartilage thickness loss at 52 weeks was less in sprifermin treated than in placebo treated knees, with effects being significant in LFTC (p=0.03) but not MFTC (p=0.16) (Table 1). Significant treatment effects ($p<0.05$) were detected in 2/16 subregions (minimal p-value =0.006 in the external lateral femur), and in 10/16 OVs (minimal p=0.004 in OV12). The 10 OVs included some in which cartilage loss was observed (OV4-8) and some in which cartilage thickening was observed in the placebo group (OV11-15).
Conclusions: This location-independent analysis shows that sprifermin can modify the magnitude of cartilage loss in subregions with low OVs, where (individual) mechanical challenges may be greater and drug effects may be more important clinically. The OV approach also has the advantage that no single region must be defined a priori as structural endpoint, which is challenging given spatial inter-subject heterogeneity of (subregional) cartilage loss in OA, particularly in cohorts without predefined (medial or lateral) involvement. The results further show that OVs are more effective and informative in revealing structural treatment effects than region-based analysis, and that sprifermin not only increases cartilage thickness (in regions where no loss is observed), but actually reduces cartilage loss (in regions where cartilage loss is observed with placebo).

Table 1
Cartilage thickness change (mm) in sprifermin treated vs. placebo knees

	Placebo Mean ± SD change	100μg dose Mean ± SD change	Difference Effect-Size	p value
MFTC	-0.07 ± 0.18	0.00 ± 0.16	0.43	0.1603
LFTC	-0.04 ± 0.19	0.04 ± 0.12	0.58	0.0323
SR^{max}	-0.03 ± 0.11	0.04 ± 0.09	0.78	0.0055
OV1	-0.22 ± 0.16	-0.18 ± 0.15	0.25	0.3529
OV2	-0.15 ± 0.12	-0.12 ± 0.12	0.25	0.3500
OV3	-0.12 ± 0.09	-0.09 ± 0.09	0.39	0.1571
OV4	-0.10 ± 0.08	-0.05 ± 0.07	0.64	0.0198
OV5	-0.08 ± 0.07	-0.04 ± 0.06	0.73	0.0094
OV6	-0.07 ± 0.06	-0.02 ± 0.06	0.79	0.0057
OV7	-0.05 ± 0.06	-0.01 ± 0.06	0.69	0.0132
OV8	-0.03 ± 0.06	0.01 ± 0.05	0.62	0.0244
OV9	-0.01 ± 0.06	0.02 ± 0.05	0.50	0.0708
OV10	0.01 ± 0.06	0.03 ± 0.05	0.49	0.0739
OV11	0.02 ± 0.06	0.05 ± 0.05	0.64	0.0210
OV12	0.03 ± 0.07	0.06 ± 0.04	0.80	0.0044
OV13	0.04 ± 0.06	0.08 ± 0.05	0.78	0.0065
OV14	0.07 ± 0.07	0.10 ± 0.05	0.64	0.0232
OV15	0.09 ± 0.07	0.13 ± 0.06	0.60	0.0305
OV16	0.15 ± 0.10	0.18 ± 0.07	0.35	0.2124

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MALALIGNMENT; A POSSIBLE TARGET FOR PREVENTION OF INCIDENT KNEE OA IN MIDDLE-AGED OVERWEIGHT AND OBESE WOMEN

J. Runhaar [†], M. van Middelkoop [†], M. Reijman [†], D. Vroegindeweij [‡], E.H. Oei [†], S.M. Bierma-Zeinstra [†]. [†] *Erasmus MC, Rotterdam, Netherlands;* [‡] *Maasstad Hosp., Rotterdam, Netherlands*

Purpose: The present study evaluates the effects of malalignment and its interaction with BMI on the onset of clinical and radiographic knee osteoarthritis (OA) over a 2.5 year follow-up period in a high risk group of middle-aged overweight and obese women.
Methods: Data of the PROOF study (ISRCTN 42823086) were used. In total, 407 women between 50 and 60 years, with a BMI ≥ 27 kg/m2, and without clinical and radiological knee OA at baseline were included in this study. Both knees of all 351 women (86%) with baseline knee alignment data and the primary outcome available were selected. At baseline, body weight and height were measured and standardized semi-flexed PA radiographs of both knees were taken according to the MTP protocol. All subjects filled in a questionnaire with questions on knee complaints and number of days with knee pain. All measurements were repeated after 2.5 years of follow-up. Minimal joint space width (medial and lateral), K&L grade and anatomical knee alignment angle were digitally assessed on all radiographs. Varus alignment was defined as an anatomical angle 184°. The predefined primary outcome measure was the incidence of knee OA, defined as onset of K&L ≥ 2 or the onset of clinical knee OA (according to the ACR criteria), or joint space narrowing (JSN) ≥ 1.0 mm in the medial or lateral compartment. Using Generalized Estimated Equations, which takes into account the correlation between knees within subjects, effects of varus and valgus alignment on the primary outcome and on the items separately were evaluated, with neutrally aligned knees serving as reference. The interaction between malalignment and baseline BMI was also studied, by adding BMI and the interaction term with malalignment to the analysis. If a significant interaction was found, overweight (BMI < 30 kg/m2) and obese subjects (BMI ≥ 30 kg/m2) were analysed separately. All analyses were adjusted for K&L grade at baseline and the randomized groups of the interventions of the PROOF study.
Results: Varus alignment was found in 40% and valgus alignment in 13% of all knees. Baseline characteristics are presented in Table 1. Overall, only varus alignment had a significant effect on the incidence of K&L ≥ 2 (9% vs. 3% in neutral knees. OR 2.8, 95% CI 1.3 - 5.9). For the primary outcome and for medial JSN a significant interaction between malalignment and baseline BMI was found ($p < 0.01$). In obese subjects, varus alignment had a significant effect on the primary outcome (22% vs. 13% in neutral knees. OR 1.8, 95% CI 1.1 - 3.1) and on medial JSN (9% vs. 4% in neutral knees. OR 2.6, 95% CI 1.1 - 6.3). These associations were not found in non-obese subjects.
Conclusions: In women at high risk for developing knee OA, varus aligned knees had a significant increased risk for the development of radiographic knee OA. Within obese women, varus aligned knees also had a significantly increased risk for incidence of knee OA according to the primary outcome and for joint space narrowing in the medial compartment. Since varus alignment is a potentially modifiable factor, results from the present study suggest that varus alignment might be a target for the prevention of knee OA in middle-aged overweight and obese women.

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DOES CARTILAGE THICKNESS CHANGE DIFFER BETWEEN ACL DEFICIENT KNEES WITH AND WITHOUT RECONSTRUCTION SURGERY

W. Wirth [†], F. Eckstein [†], M. Hudelmaier [†], S. Lohmander [‡], R. Frobell [‡]. [†] *Paracelsus Med. Univ., Salzburg, Austria;* [‡] *Orthopedics, Clinical Sci. Lund, Lund Univ., Lund, Sweden*

Purpose: An ACL tear is a common knee injury, involving a serious trauma and a subsequent period of chronic alterations in joint biomechanics. The risk of developing incident knee OA after an ACL tear is known to be highly elevated although the driving mechanisms are not known. In this study, we tested the hypothesis that treatment of the initial injury influenced change in femorotibial cartilage thickness over the first five years after injury. We thus explored femorotibial cartilage thickness changes during the first 2 years (BL→Y2) and during a subsequent three-year period (Y2→Y5) after an acute ACL tear.